The authors have no relevant conflicts of interest

FUNCTIONAL ANALYSIS OF NOVEL *ABCC8* MUTATIONS FOUND IN CZECH PATIENTS WITH CONGENITAL HYPERINSULINISM

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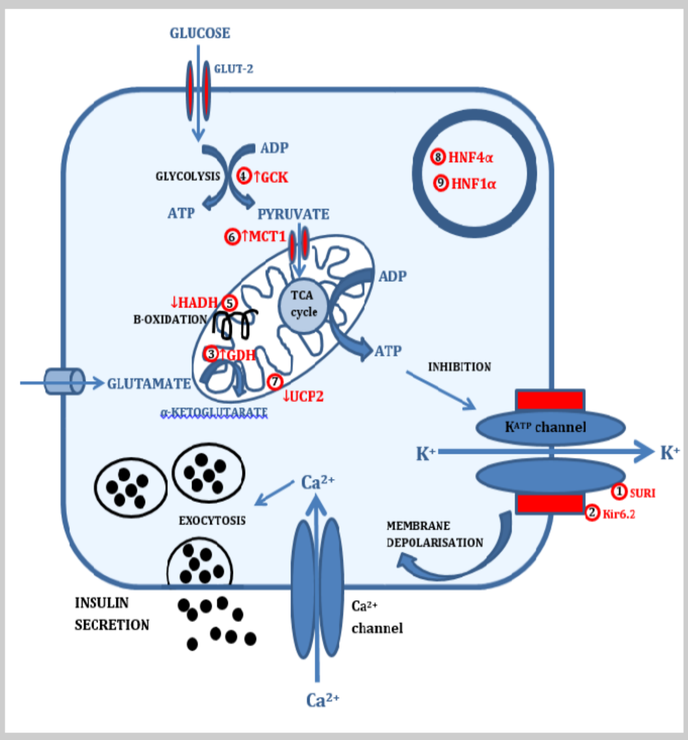
Background

- Congenital Hyperinsulinism (CHI) is a heterogeneous genetically determined condition that is characterized by unregulated secretion of insulin from pancreatic β-cells. The most common and severe cases are caused by mutations in K_{ATP} channel subunit SUR1 encoded by the gene *ABCC8*. To assess the pathogenic effect of novel *ABCC8* mutations it is necessary to perform in-vitro functional study.

Objective and hypotheses

- The aim of our study was to identify Czech patients with CHI caused by *ABCC8* mutations and to perform in-vitro functional study of novel *ABCC8* mutations found in our cohort.



Genes involved in the pathogenesis of CHI

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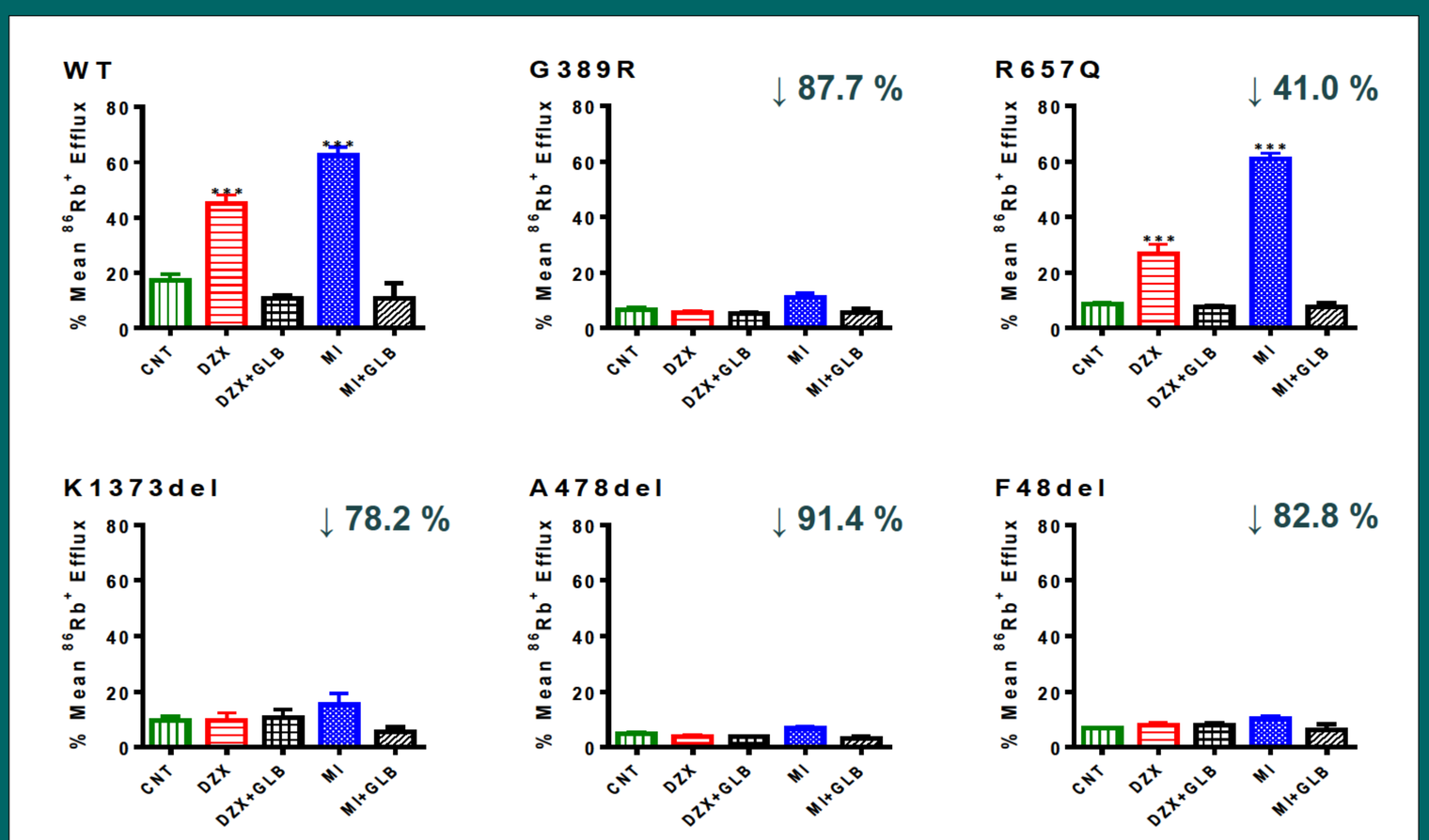
Methods

- The molecular genetic analysis of *ABCC8* gene was performed on DNA samples of 42 Czech patients with CHI. Novel mutations were created by site directed mutagenesis and transfected into HEK293 cells for functional studies using radioactive Rubidium ($^{86+}$Rb). Mutant and wild type (WT) channels were exposed to different drug conditions: control (DMSO), 100μM diazoxide, 100μM diazoxide and 10μM glibenclamide, 2.5mM NaCN and 20mM 2-deoxy-D-glucose and 2.5mM NaCN, 20mM 2-deoxy-D-glucose and 10μM glibenclamide. $^{86+}$Rb efflux was measured in a liquid scintillation counter using Cherenkov radiation.

Results



Determining the functional impact of SUR1 mutations (G389R, R657Q, K1373del, A478del, F48del) on K_{ATP} channel function using the $^{86+}$Rb efflux

Results

Mutations in *ABCC8* were identified in 12 out of 42 patients (28.6 %) – 1 homozygous and 11 heterozygous (5 novel: F48del, G389R, A478del, R657Q, and K1373del). The $^{86+}$Rb efflux assay showed that in mutant channels the activation by diazoxide was decreased by 41-91.4 % (median 82.2 %) when compared to WT channels. The most severe effect on K_{ATP} channel function was observed in case of A478del (activity decreased by 91.4 %). On the other hand in case of R657Q there was a residual activation by diazoxide in correspondence with the patient's phenotype (activity decreased by 41 %).

Conclusion

We report the biggest cohort of Czech patients with CHI published so far. The proportion of heterozygous mutations is much higher when compared to other published cohorts, most probably due to lack of consanguinity in the Czech population. Moreover, using in-vitro functional study, we have proved the pathogenic effect of 5 novel heterozygous *ABCC8* mutations on the pancreatic K_{ATP} channel function.

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